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Oxygen Is the High-Energy Molecule Powering Complex Multicellular Life: Fundamental Corrections to Traditional Bioenergetics

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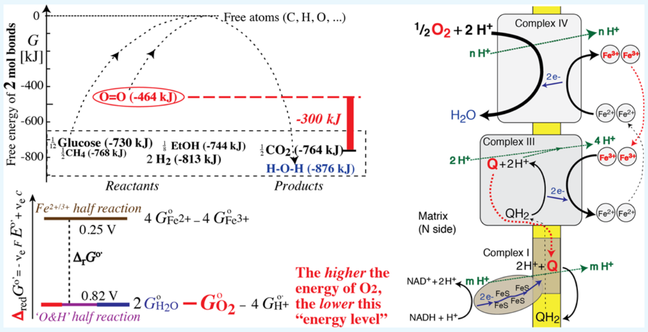
ABSTRACT: A fundamental re-assessment of the overall energetics of biochemical electron transfer chains and cycles is presented, highlighting the crucial role of the highest-energy molecule involved, O_2. The chemical energy utilized by most complex multicellular organisms is not predominantly stored in glucose or fat, but rather in O_2 with its relatively weak (i.e., high-energy) double bond. Accordingly, reactions of O_2 with organic molecules are highly exergonic, while other reactions of glucose, fat, NAD(P)H, or ubiquinol (QH_2) are not, as demonstrated in anaerobic respiration with its meager energy output. The notion that "reduced molecules" such as alkanes or fatty acids are energy-rich is shown to be incorrect; they only unlock the energy of more O_2, compared to O-containing molecules of similar mass. Glucose contains a moderate amount of chemical energy per bond (<20% compared to O_2), as confirmed by the relatively small energy output in glycolysis and the Krebs cycle converting glucose to CO_2 and NADH. Only in the "terminal" aerobic respiration reaction with O_2 does a large free energy change occur due to the release of oxygen's stored chemical energy. The actual reaction of O_2 in complex IV of the inner mitochondrial membrane does not even involve any organic fuel molecule and yet releases >1 MJ when 6 mol of O_2 reacts. The traditional presentation that relegated O_2 to the role of a low-energy terminal acceptor for depleted electrons has not explained these salient observations and must be abandoned. Its central notion that electrons release energy because they move from a high-energy donor to a low-energy acceptor is demonstrably false. The energies of (at least) two donor and two acceptor species come into play, and the low "terminal" negative reduction potential in aerobic respiration can be attributed to the unusually high energy of O_2, the crucial reactant. This is confirmed by comparison with the corresponding half-reaction without O_2, which is endergonic. In addition, the electrons are mostly not accepted by oxygen but by hydrogen. Redox energy transfer and release diagrams are introduced to provide a superior representation of the energetics of the various species in coupled half-reactions. Electron transport by movement of reduced molecules in the electron transfer chain is shown to run counter to the energy flow, which is carried by oxidized species. O_2, rather than glucose, NAD(P)H, or ATP, is the molecule that provides the most energy to animals and plants and is crucial for sustaining large complex life forms. The analysis also highlights a significant discrepancy in the proposed energetics of reactions of aerobic respiration, which should be re-evaluated.



■ INTRODUCTION

Bioenergetics is an important area of biochemistry, accounting for the energy driving biochemical reactions.[1−8] While it is known, in principle, that only reactions with molecular oxygen provide enough energy to make large complex organisms viable,[9] biochemistry and biology textbooks assume without proof that biochemical energy is stored in fuel molecules such as glucose.[1−5,8,10,11] In this paper, we demonstrate that this view is incorrect since most of the energy is actually derived from O_2 with its relatively weak double bond.[12]

The fact that most chemical energy is associated with chemical bonds is acknowledged early on in many biochemistry texts[1,2,4,5,8] and emphasized in the context of adenosine triphosphate (ATP). Molecules with relatively weak bonds store chemical energy and release it when chemical reactions result in the formation of the same number of stronger, lower-energy bonds. Since, according to the traditional view, fuel molecules such as glucose or fat are full of energy, a significant fraction of their stored energy should be released when they decompose. However, compared to the combustion reaction

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$$\begin{gathered} C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O \\ \Delta_r H^o = -2805\ \text{kJ/mol},\ \Delta_r G^o = -2875\ \text{kJ/mol} = \Delta_r G^{o\prime\prime} \end{gathered} \tag{1}$$

decompositions of fuel molecules are not strongly exergonic:

$$\begin{gathered} C_6H_{12}O_6 \rightarrow 2C_3H_5O_3^{-}(\text{lactate}) + 2H^+ \\ \Delta_r H^o = -109\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = -198\ \text{kJ/mol} \end{gathered} \tag{2a}$$

$$\begin{gathered} C_6H_{12}O_6 \rightarrow 3CH_3COOH(\text{acetic acid}) \\ \Delta_r H^o = -194\ \text{kJ/mol},\ \Delta_r G^o = -271\ \text{kJ/mol} \end{gathered} \tag{2b}$$

$$\begin{gathered} C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \\ (\text{alcoholic fermentation}) \\ \Delta_r H^o = -100\ \text{kJ/mol},\ \Delta_r G^o = -235\ \text{kJ/mol}, \\ \Delta_r G^{o\prime\prime} = -236\ \text{kJ/mol} \end{gathered} \tag{2c}$$

$$\begin{gathered} C_6H_{12}O_6 \rightarrow 6C + 6H_2O \\ \Delta_r H^o = -441\ \text{kJ/mol},\ \Delta_r G^o = -511\ \text{kJ/mol} \end{gathered} \tag{2d}$$

$$\begin{gathered} C_6H_{12}O_6 \rightarrow 6C + 6H_2 + 3O_2 \\ \Delta_r H^o = +1275\ \text{kJ/mol}, \\ \Delta_r G^o = +911\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = +918\ \text{kJ/mol} \end{gathered} \tag{2e}$$

$$\begin{gathered} C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \\ \Delta_r H^o = -132\ \text{kJ/mol},\ \Delta_r G^o = -424\ \text{kJ/mol} \end{gathered} \tag{2f}$$

$$\begin{gathered} C_{15}H_{31}COOH(\text{fat}) \rightarrow 2C_6H_6 + CO_2 + 3CH_4 + 4H_2 \\ \Delta_r H^o = +296\ \text{kJ/mol},\ \Delta_r G^o = +8\ \text{kJ/mol} \end{gathered} \tag{2g}$$

$$\begin{gathered} C_{15}H_{31}COOH \rightarrow 16C + 16H_2 + O_2 \\ \Delta_r H^o = +892\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = +308\ \text{kJ/mol} \end{gathered} \tag{2h}$$

Double-primed quantities were calculated under Alberty's biological standard conditions (at pH = 7);[13,14] the relatively small differences from the unprimed values[7,15] or the single-primed values at pH 7[16] are mostly entropic in nature and inconsequential for our conclusions. Reactions of fuel molecules in the absence of O_2 are also not strongly exergonic, even when a strongly bonded molecule such as CO_2 is formed:

$$\begin{gathered} C_6H_{12}O_6 + 2NAD^+ \rightarrow 2CH_3(C{=}O)COO^-(\text{pyruvate}) \\ + 2NADH + 4H^+ \\ \Delta_r G^{o\prime} = -147\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = -153\ \text{kJ/mol} \end{gathered} \tag{3a}$$

$$\begin{gathered} C_6H_{12}O_6 + 6NADH + 6H^+ \rightarrow 6NAD^+ + 6CH_4 + 3O_2 \\ \Delta_r G^{o\prime} = +707\ \text{kJ/mol} \end{gathered} \tag{3b}$$

$$\begin{gathered} 6CH_4 + 12H_2O \rightarrow 6CO_2 + 24H_2 \\ (\text{methanogens in reverse}) \\ \Delta_r H^o = +1518\ \text{kJ/mol},\ \Delta_r G^o = +786\ \text{kJ/mol}, \\ \Delta_r G^{o\prime\prime} = +804\ \text{kJ/mol} \end{gathered} \tag{3c}$$

$$\begin{gathered} C_{15}H_{31}COOH + 30H_2O \rightarrow 16CO_2 + 46H_2 \\ \Delta_r H^o = +3168\ \text{kJ/mol},\ \Delta_r G^o = +1111\ \text{kJ/mol}, \\ \Delta_r G^{o\prime\prime} = +1148\ \text{kJ/mol} \end{gathered} \tag{3d}$$

(for $\Delta_r G^{o\prime}$ in eq 3a, see ref 16). Other reactions of glucose shown below have a similarly meager energy output. By contrast, when organic molecules react with O_2, invariably a lot of energy is released:

$$\begin{gathered} 2C_2H_5OH + 6O_2 \rightarrow 4CO_2 + 6H_2O \\ \Delta_r G^o = -2648\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = -2638\ \text{kJ/mol} \end{gathered} \tag{4a}$$

$$\begin{gathered} 12NADH + 12H^+ + 6O_2 \rightarrow 12NAD^+ + 12H_2O \\ \Delta_r G^{o\prime} = -2631\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = -2598\ \text{kJ/mol} \end{gathered} \tag{4b}$$

$$\begin{gathered} 2C_2H_7NCO_2(\text{alanine}) + 6O_2 \rightarrow O{=}C(NH_2)_2\ (\text{urea}) \\ + 5CO_2 + 5H_2O \quad \Delta_r G^{o\prime\prime} = -2618\ \text{kJ/mol} \end{gathered} \tag{4c}$$

$$\begin{gathered} 6C_2H_5OH + 6O_2 \rightarrow 2C_6H_{12}O_6 + 6H_2O \\ \Delta_r G^o = -2152\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = -2168\ \text{kJ/mol} \end{gathered} \tag{4d}$$

$$\begin{gathered} 12C_2H_5OH + 6O_2 \rightarrow 8C_3H_3O_3^{-}(\text{pyruv.}) + 8H^+ \\ +20H_2 \quad \Delta_r G^{o\prime\prime} = -1934\ \text{kJ/mol} \end{gathered} \tag{4e}$$

It is apparent from these examples and others given below that more O_2 in the reaction results in the release of more energy,[12,17−21] almost regardless of the nature of the fuel molecules or reaction products. Note that several of the reactions with O_2 shown, as well as additional examples below, do not produce CO_2 and yet are significantly exergonic. The obvious interpretation of these observations is that a lot of chemical energy resides in O_2.[12,20,21] We had previously shown, through a generalized bond-energy analysis, that 418 kJ of heat is released per mole of O_2 in the combustion of organic molecules.[12] The excellent agreement of this analysis with the experimental data has convincingly documented its validity.[12] O_2 provides ~3/4 of the energy of combustion due to its relatively weak σ-bonding (while the π-electrons are complicated but provide strong bonding);[22] the organic fuels contribute only ~1/4.[12]

While the generalized bond-energy analysis[12] has clearly proven that O_2 contains most of the chemical energy in the biosphere, the established presentation of bioenergetics[1−8,11] suggests the opposite. Energy diagrams commonly show nutrients, in addition to sunlight, as the energy sources of organisms and do not mention oxygen (see the Supporting Information for examples).[1−5,8] "Reduced molecules" are the supposed energy-rich species.[1,5,6] In respiration, O_2 is often called the "terminal electron receptor"; it is shown with a low "energy level" (negative of the standard reduction potential),[6] which suggests a low-energy species, essentially a receptacle for "used electrons".[23] The main goal of this paper is to show how the commonly invoked electron transfer picture[1−3,6] can be reconciled with O_2 being the highest-energy molecule (per bond) commonly found in the biosphere and how to correctly analyze the overall energetics of electron transfer chains and the Krebs and Calvin cycles.

Our analysis focuses on aerobic respiration since its equivalence to combustion, already quantitatively analyzed and explained,[12] makes it easier to recognize the special role of O_2 as the main high-energy molecule in the biosphere. Respiration processes will be presented in diagrams that correctly reflect the flow of chemical energy from O_2. It is shown that the new analysis is not just an optional alternative view but that the traditional reasoning is incorrect and must be replaced. Recognizing O_2 as the crucial high-energy molecule also highlights discrepancies between free energy release and claimed ATP production in different reactions of aerobic respiration.

■ RESULTS AND DISCUSSION

Energetics of Molecules in Aerobic Respiration.

Summary of the Traditional Presentation. We had previously established that O_2 is the most important high-energy molecule in the biosphere,[12] storing large amounts of solar energy. What we analyze here is why this fact has not been apparent in traditional descriptions of bioenergetics[1−8,11] and how the presentation needs to be changed.

Textbooks describe biochemical energetics at three main levels:

(i) Conceptual overviews of bioenergetics show naïve statements and diagrams about sunlight and nutrients providing the energy organisms need,[1,2,8] and it is assumed without reflection that organic fuel molecules contain the energy released in aerobic respiration.[3−5,11]

(ii) In more specific summaries of respiration and photosynthesis reactions, the Gibbs free energy changes $\Delta_r G^{o\prime}$ in overall reactions, such as $\mathrm{NADH} + \mathrm{H}^+ + 1/2\mathrm{O}_2 \rightarrow \mathrm{H_2O} + \mathrm{NAD}^+$, are given but without meaningful explanation. (We have shown that their main component, $\Delta_r H^o$, can be quantitatively explained in terms of the energy per electron-pair bond.[12] In combustion and aerobic respiration, most of the energy derives from the unusually weak double bond of O_2.)

(iii) An electron transfer description is given at length.[1−3,6] In the description of the electron transfer step by step, the origin of the large $\Delta_r G^{o\prime}$ in aerobic respiration is not discussed. This analysis does not even explicitly refer to energies but rather to negative standard reduction potentials in volts. It is "explained" in terms of differential electron affinities or an "electron waterfall".[6,24]

If students combine these concepts, they may reasonably (but incorrectly) conclude that the energy of combustion mostly derives from the bond energies of organic "fuel" molecules and that the energy differences are due to different electron affinities; for instance, the final step of aerobic respiration with transfer of electrons to oxygen is interpreted as the low-energy endpoint of the sequence of reactions.[1,3,6] In what ways these conclusions are incorrect will be explained in the following.

Energy Released in Aerobic Respiration. The respiration of glucose is summarized by the familiar reaction in eq 1 with $\Delta_r G^o = -2875$ kJ/mol, and that of a fatty acid (palmitic acid) by

$$\mathrm{C_{15}H_{31}COOH + 23O_2 \rightarrow 16H_2O + 16CO_2}$$
$$\Delta_r H^o = -9978\ \mathrm{kJ/mol},\ \Delta_r G^{o\prime\prime} = -9803\ \mathrm{kJ/mol} \quad (5)$$

while the overall reaction in the "electron transfer chain of oxidative phosphorylation"[1] is

$$\mathrm{12NADH + 12H^+ + 6O_2 \rightarrow 12NAD^+ + 12H_2O}(l)$$
$$\Delta_r G^{o\prime} = -2640\ \mathrm{kJ/mol},\ \Delta_r G^{o\prime\prime} = -2600\ \mathrm{kJ/mol} \quad (6)$$

All three reactions are highly exothermic and exergonic, which means that a lot of chemical energy must have been stored in the bonds of the reactants that are broken during the reaction (much more, for instance, than in the P−O bonds of 12 ATP molecules). While the traditional view has been that the energy is stored in glucose or NADH + H^+, we will show in the following that glucose and NADH are, at best, moderate-energy species, and "fully reduced" hydrocarbons are low-energy molecules in the absence of O_2. Instead, O_2 provides $\geq 3/4$ of the energy released[12] in respiration and the "electron transfer chain".

High-Energy Molecules. Chemical reactions releasing energy typically involve multiple reactants. In examples such as the reactions in eqs 1, 5, and 6, it may not be clear a priori which of the reactants provides the energy. In addition, the relative energy of the products also plays an important role. Nevertheless, a molecule or chemical species can be considered to have high energy if it releases hundreds of kilojoules per mole of bonds broken and reformed in reactions with a wide range of other reactants and generating various products.

High-energy molecules have relatively weak bonds; when these are broken and converted to stronger bonds in the products, energy is released. It must be noted that such an energy analysis in terms of weak bonds converting to stronger bonds can be generalized easily only if the number of bonds remains unchanged (see examples in the Supporting Information). This applies only to electron-pair bonds.[12] Therefore, when speaking about weak bonds and strong bonds in the context of chemical energy, we should count a double bond as two bonds.[12]

O_2 Is a High-Energy Molecule. The reaction of O_2 with an organic molecule can be written as

$$\mathrm{C}_c\mathrm{O}_o\mathrm{H}_h\mathrm{N}_n + \nu_{\mathrm{O_2}}\,\mathrm{O_2}$$
$$\rightarrow \nu_{\mathrm{H_2O}}\,\mathrm{H_2O}(l) + \nu_{\mathrm{CO_2}}\,\mathrm{CO_2} + \nu_{\mathrm{N_2}}\,\mathrm{N_2} \quad (7)$$

This represents thousands of different strongly exothermic reactions. A generalized bond energy analysis has shown that $\Delta_c H^o = \nu_{\mathrm{O_2}}\,(-418 \pm 12\ \mathrm{kJ/mol})$ is released,[12] in other words, that the heat of combustion is proportional to the amount of O_2 used up. The proportionality of $\Delta_c H^o$ with $\nu_{\mathrm{O_2}}$ was discovered empirically several times,[17,18] including in the context of biochemistry.[19] The energy can be attributed to the relatively weak double bond of O_2, which is 300 kJ/mol higher than that of a double bond in CO_2, about 400 kJ/mol higher than the two bonds in H_2O, and 200−350 kJ/mol higher than pairs of single bonds of C in organic fuels.[12,20,21] The bonding in O_2, with a σ-bond even weaker than in H_2O_2 or F_2 and a complicated but significantly bonding π-electron system,[22] is reviewed in the Supporting Information. A bond energy analysis demonstrating that O_2 is a high-energy molecule irrespective of its bond order can be found in the Supporting Information.

The entropic contribution to the free energy of combustion at $T = 298$ K derived in the Supporting Information is usually a fairly small correction to $\Delta_c H^o = -418\ \mathrm{kJ/mol}\ (c + 0.3h - 0.5o)$.[12] Overall, we obtain

$$\Delta_c G^o = \Delta_c H^o - T\Delta_c S^o$$
$$\approx -418\ \mathrm{kJ/mol}(c + 0.314h - 0.43o + 0.07n)$$
$$+ 298\ \mathrm{K}\ S^o{}_{\mathrm{fuel}} \quad (8)$$

If the standard molar entropy of the fuel is not known, then $S^o{}_{\mathrm{fuel}} = 200$ J/(mol K) is often a sufficiently good approximation for fairly small molecules. For glucose, the equation predicts the free energy of combustion with better than 3% accuracy. The energetics of combustion are visualized in Figure 1a, which shows the average free energy of a double bond or two single bonds in molecules relevant in respiration. The special role of O_2 as a high-energy molecule is similarly apparent here as in the analogous plot of bond-formation enthalpies.[12]

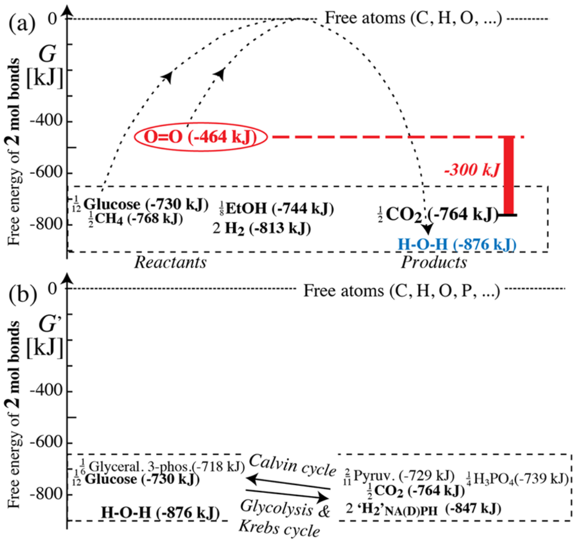

Figure 1. Free energy of a mole of double bonds or 2 mol of single bonds in various molecules involved in important biochemical processes. The more stable a bonded species is, the lower it appears in the diagram. (a) Free energy diagram for combustion or respiration. For glucose and ethanol, the graph shows the average free energies of 2 mol of bonds, that is, 1/12 and 1/8, respectively, of the total bonding free energy listed in Table S1. Similar values are found for 2 mol of bonds in methanol (−756 kJ) and in acetic acid (−754 kJ); see the Supporting Information. (b) Visualization of the nearly unchanged energetics in the Calvin cycle and in glycolysis plus the Krebs cycle in terms of the average free energy of 2 mol of electron pair bonds. "H_2"$_{\mathrm{NAD(P)H}}$ represents the energy of NAD(P)H + H^+ relative to NAD(P)$^+$, which can be considered a H_2 analogue (see the Supporting Information for details). Pyruv., pyruvic acid; Glyceral. 3-phos., glyceraldehyde 3-phosphate. All species with labile protons are assumed to be fully protonated for simplicity.

In addition to reactions in eqs 4a–4e and 7, other instructive reactions of O_2 include

$$12H_2 + 6O_2 \rightarrow \mathbf{12H_2O} \quad \Delta_rG^\circ = -2844\ \text{kJ/mol} \tag{9a}$$

$$6C + 6O_2 \rightarrow \mathbf{6CO_2} \quad \Delta_rG^\circ = -2364\ \text{kJ/mol} \tag{9b}$$

$$14.4CH_4 + 6O_2 \rightarrow 2.4C_6H_{14} + 12H_2O \quad \Delta_rG^\circ = -2120\ \text{kJ/mol} \tag{9c}$$

$$6C_2H_5OH + 6O_2 \rightarrow 6CH_3COOH + 6H_2O \quad \Delta_rG^\circ = -2712\ \text{kJ/mol} \tag{9d}$$

$$6H_2C{=}CH_2 + 6O_2 \rightarrow \mathbf{6CH_3COOH} \quad \Delta_rG^\circ = -2748\ \text{kJ/mol} \tag{9e}$$

$$12H_3C{-}CH_3 + 6O_2 \rightarrow \mathbf{12CH_3CH_2OH} \quad \Delta_rG^\circ = -1714\ \text{kJ/mol} \tag{9f}$$

$$2C_6H_{14} + 6O_2 \rightarrow \mathbf{2C_6H_{12}O_6} + \mathbf{2H_2} \quad \Delta_rG^\circ = -1830\ \text{kJ/mol} \tag{9g}$$

$$10C + 2.5N_2 + 8H_2 + 6.5O_2 + 3P \rightarrow \mathbf{ATP} \quad \Delta_rG^{\circ\prime\prime} = -2950\ \text{kJ/mol} \tag{9h}$$

$$24Cr^{2+}(aq) + 24H^+(aq) + 6O_2 \rightarrow 24Cr^{3+}(aq) + 12H_2O(l) \quad \Delta_rG^\circ = -2826\ \text{kJ/mol} \tag{9i}$$

$$12Fe(s) + 24H^+(aq) + 6O_2 \rightarrow 12Fe^{2+}(aq) + 12H_2O(l) \quad \Delta_rG^{\circ\prime} = -2849\ \text{kJ/mol},\ \Delta_rG^\circ = -3800\ \text{kJ/mol} \tag{9j}$$

These examples, all balanced with ~6 O_2 to match the combustion of glucose in eq 1, show that reactions with O_2 release a lot of energy, regardless of whether or not the products contain CO_2 or H_2O. This qualifies O_2 as a high-energy molecule, consistent with Figure 1a.

Glucose Has Only Moderate Energy. Unlike O_2, glucose does not release a lot of energy (per mass or per bond) in many of its reactions. Examples of this, including alcoholic and lactic acid fermentation, are shown in eqs 2a–2f, 3a, and 3b. Here, we add several more:

$$C_6H_{12}O_6 \rightarrow 6H_2C{=}O \quad \Delta_rH^\circ = +580\ \text{kJ/mol},\ \Delta_rG^\circ = +251\ \text{kJ/mol} \tag{10a}$$

$$C_6H_{12}O_6 \rightarrow 6CO + 6H_2 \quad \Delta_rH^\circ = +612\ \text{kJ/mol},\ \Delta_rG^\circ = +89\ \text{kJ/mol} \tag{10b}$$

$$C_6H_{12}O_6 \rightarrow C_6H_6 + 3H_2O_2 \quad \Delta_rH^\circ = +916\ \text{kJ/mol},\ \Delta_rG^\circ = +719\ \text{kJ/mol} \tag{10c}$$

$$C_6H_{12}O_6 \rightarrow 2C_3H_6O_3(\text{glyceraldehyde}) \quad \Delta_rG^{\circ\prime\prime} \approx +30\ \text{kJ/mol} \tag{10d}$$

$$C_6H_{12}O_6 \rightarrow 2.5CO_2 + 1/2C_6H_5COOH + 4.5H_2 \quad \Delta_rG^\circ = -197\ \text{kJ/mol} \tag{10e}$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \quad \Delta_rH^\circ = +627\ \text{kJ/mol},\ \Delta_rG^\circ = -31\ \text{kJ/mol} \tag{10f}$$

$$C_6H_{12}O_6 + 6H_2O + 10NAD^+ + 2FAD \rightarrow 10NADH + 10H^+ + 2FADH_2 + 6CO_2 \quad \Delta_rG^{\circ\prime} = -341\ \text{kJ/mol},\ \Delta_rG^{\circ\prime\prime} = -307\ \text{kJ/mol} \tag{10g}$$

(for the data needed to calculate Δ_rG° in eqs 10a and 10c, see ref 25).

The data show that reactions of glucose without O_2 release only a fraction of the free energy given off in the glucose combustion reaction in eq 1. This is can be understood in terms of average bond free energies (see Figure 1), which are much lower for glucose than for O_2 but slightly higher than for some of the other molecules involved in the reactions. It should also be noted that $\Delta_r G^o$ contains up to −650 kJ/mol not from bond energies but from $-T\Delta_r S^o$: there is an entropy increase due to the breakup of a larger molecule such as glucose into several small ones (with typical molar entropies of 70 to 215 J/(mol K), corresponding to −21 to −64 kJ/mol in $\Delta_r G^o$ at 298 K).

The fact that glucose is not a high-energy molecule is highlighted by the fairly minor energy release upon its conversion into smaller molecules (see eqs 2a−2f and 10a−10d), including some producing CO_2 with its strong, 804 kJ/mol bonds. When the bonds of glucose are broken, the energy stored in these bonds should be released; less than 30 kJ per mole of bonds is observed, while O_2 gives off 300 kJ/mol when its double bond is converted to a double bond in CO_2.[12]

Reduced Molecules Have Little Energy. Hydrocarbons are excellent fuels in combustion because they react with many O_2 molecules and unlock their energy.[12] Their large energy of combustion per mass has resulted in the lore[1,2,5] that these "highly reduced molecules" are energy-rich. However, the "most reduced molecule", CH_4, does not release energy when it breaks up or reacts

$$6CH_4 \rightarrow 6C(s) + 12H_2 \quad \Delta_r H^o = +449\ \text{kJ/mol},\ \Delta_r G^o = +306\ \text{kJ/mol} \tag{11a}$$

$$6CH_4 \rightarrow C_6H_{14} + 5H_2 \quad \Delta_r H^o = +649\ \text{kJ/mol},\ \Delta_r G^o = +302\ \text{kJ/mol} \tag{11b}$$

$$6CH_4 \rightarrow C_6H_6 + 9H_2 \quad \Delta_r H^o = +499\ \text{kJ/mol},\ \Delta_r G^o = +430\ \text{kJ/mol} \tag{11c}$$

$$CH_4 + 6H_2O \rightarrow 2CH_3(C{=}O)COOH + 14H_2 \quad \Delta_r G^o = +750\ \text{kJ/mol} \tag{11d}$$

$$6CH_4 + 6H_2O \rightarrow C_6H_{12}O_6 + 12H_2 \quad \Delta_r H^o = +889\ \text{kJ/mol},\ \Delta_r G^o = +817\ \text{kJ/mol} \tag{11e}$$

$$6CH_4 + 6CO_2 \rightarrow 2C_6H_{12}O_6 \quad \Delta_r H^o = +264\ \text{kJ/mol},\ \Delta_r G^o = +848\ \text{kJ/mol} \tag{11f}$$

$$15CH_4 + CO_2 \rightarrow C_{15}H_{31}COOH + 14H_2 \quad \Delta_r G^{o\prime\prime} = +859\ \text{kJ/mol} \tag{11g}$$

except when reacting with O_2 or other oxidants with weak bonds. This is consistent with the low energy of CH_4 due to strong bonding (see Figure 1a). Indeed, certain anaerobic microbes, the methanogens, power themselves by running the reaction in eq 3c in reverse, which takes advantage of the low energy of methane and water as products.[26] The *n*-alkanes and fatty acids show similarly little energy release except when reacting with O_2 (see the Supporting Information). This disproves the claim that reduced molecules contain a lot of energy.

NAD(P)H Is Not a High-Energy Molecule. Based on the standard presentation of bioenergetics,[3,6] it is easy to conclude that "the bulk of the required energy comes from NADPH"[27] or that "NADPH is an energy-carrying molecule produced in the first stage of photosynthesis. It provides energy to fuel the Calvin cycle in the second stage of photosynthesis".[28] One can show that this is erroneous by analyzing the energetics of reactions of nicotinamide adenine dinucleotide (phosphate) or NAD(P)H with various molecules or species (other than O_2) available in the biosphere:

$$12NAD(P)H + 12H^+ + 6CO_2 \rightarrow 12NAD(P)^+ + C_6H_{12}O_6 + 6H_2O(l) \quad \Delta_r G^{o\prime} = +235\ \text{kJ/mol},\ \ \Delta_r G^{o\prime\prime} = +269\ \text{kJ/mol} \tag{12a}$$

$$12NAD(P)H + 12H^+ + 12(CH_3)_2C{=}O \rightarrow 12NAD(P)^+ + 12(CH_3)_2CHOH \quad \Delta_r G^{o\prime\prime} = -66\ \text{kJ/mol} \tag{12b}$$

$$12NAD(P)H + 12H^+ + 12\ \text{pyruvate} \rightarrow 12NAD(P)^+ + 12\ \text{lactate} \quad \Delta_r G^{o\prime\prime} = -293\ \text{kJ/mol} \tag{12c}$$

$$12NAD(P)H + 12H^+ + 12\ \text{oxaloacetate} \rightarrow 12NAD(P)^+ + 12\ \text{malate} \quad \Delta_r G^{o\prime\prime} = -353\ \text{kJ/mol} \tag{12d}$$

$$12NAD(P)H + 12H^+ + 3CO_2 \rightarrow 12NAD(P)^+ + 3CH_4 + 6H_2O(l) \quad \Delta_r G^{o\prime} = -189\ \text{kJ/mol} \tag{12e}$$

$$NAD(P)H + H^+ \rightarrow NAD(P)^+ + H_2 \quad \Delta_r G^{o\prime} = +17\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = +21\ \text{kJ/mol} \tag{12f}$$

For ease of comparison with aerobic respiration (eq 6), most reactions have been considered for 12NAD(P)H. The data show that reactions of NAD(P)H without O_2 are sometimes endergonic and release at most one-seventh of the energy of the reaction in eq 6 with O_2. This demonstrates that NAD(P)H does not qualify as a high-energy molecule.

In the analysis of the chemical energy of the fairly complex-looking reactions in eqs 12a−12f, a simplifying approximation proved useful (ref 1, p 661): NAD(P)H + H^+ undergoes the same reactions as H_2 (see the Supporting Information for examples); when analyzing the energetics, one can consider NAD(P) as "H", a hypothetical species whose bond energy to H and ionization energy in water are similar to the corresponding energies of H. Thus, within a biochemical reaction, one can replace

$$NAD(P)H + H^+ \rightarrow NAD(P)^+ + \tag{13a}$$

with

$$\text{“H”}{-}H + H^+ \rightarrow \text{“H”}^+ \tag{13b}$$

and even shorter

$$\text{“}H_2\text{”}_{NAD(P)H} \rightarrow \tag{13c}$$

where we treat "H_2"$_{NAD(P)H}$ as a hypothetical form of H_2 with $\Delta_f G^{o\prime}{}_{\text{“H2”(NAD(P)H)}} = -17$ kJ/mol or $\Delta_f G^{o\prime\prime}{}_{\text{“H2”(NAD(P)H)}} = +61$ kJ/mol (which is at −21 kJ/mol relative to $H_2(g)$).[13,14] In this context, it is relevant to note that the bond in H_2 is relatively strong, 436 kJ/mol, similar to C−H (410 kJ/mol) and stronger than C−C or C−O (350 kJ/mol) bonds, so H_2 is not a high-energy molecule (see also Figure 1a).

Representing the Energetics of Redox Reactions in Respiration. *Two Valid Views of Energy Release in a Redox Reaction.* Two complementary views of the energetics of redox reactions can be found explicitly or implicitly in biochemistry textbooks;[1,6] both enable calculation of the standard free energy change $\Delta_r G^o$ from tabulated, measured thermodynamic quantities, but it is important not to confuse them conceptually. We demonstrate the concepts and calculations for the example of

$$\mathrm{O_2 + 4H^+(aq) + 4Fe^{2+}(cyt}c) \rightarrow \mathrm{2H_2O + 4Fe^{3+}(cyt}c)$$
$$\Delta_r G^{o\prime} = -218\ \mathrm{kJ/mol},\ \Delta_r G^{o\prime\prime} = -229\ \mathrm{kJ/mol} \tag{14}$$

where $\mathrm{Fe^{2+}(cyt}c)$ stands for a ferrous ion in cytochrome *c*. This is the most important reaction in aerobic respiration, releasing the most energy in a single step ($\Delta_r G^{o\prime} = -1308$ kJ/mol with $6O_2$, nearly half of the total of the reaction in eq 1). While this has traditionally been described as the terminal step of aerobic respiration, it can be argued that it should be regarded as the first step (see the Supporting Information).

High-Energy Reactants to Lower-Energy Products. From the free energies of products, $G^{o\prime}_{\text{prod}}$, and reactants, $G^{o\prime}_{\text{reactants}}$, pure in their standard states and weighted with the stoichiometric coefficients ν_i, $\Delta_r G^{o\prime}$ can be calculated simply as the difference

$$\begin{aligned}\Delta_r G^{o\prime} &= G^{o\prime}_{\text{prod}} - G^{o\prime}_{\text{reactants}} \\ &= (2G^o_{\mathrm{H_2O}} + 4G^o_{\mathrm{Fe^{3+}(cyt}c)}) \\ &\quad - (G^o_{\mathrm{O_2}} + 4G^{o\prime}_{\mathrm{H^+}} + 4G^o_{\mathrm{Fe^{2+}(cyt}c)})\end{aligned} \tag{15}$$

Thus, in a graph of $G^{o\prime}_{\text{prod}}$ and $G^{o\prime}_{\text{reactants}}$, $\Delta_r G^{o\prime}$ is the "gap" between the "free energy levels" (see Figure 2a and Figure S1). This visualizes that the chemical energy released in a reaction has been stored in relatively high-energy reactants and is released when lower-energy products are formed.

In the Supporting Information, it is shown how eq 15 can be evaluated either through the traditional thermochemistry approach with difficult-to-interpret free energies of formation or more meaningfully using the individual free energy G^o_i of species *i* relative to the free atoms, that is, based on bond energies. Because only the latter analysis has explanatory power, it is the one we adopt in this paper. It fits with the initial correct notion in some biochemistry textbooks that chemical energy is associated with chemical bonding. Table S1 lists meaningful individual free energies G^o_i of various molecules and ions relevant in this paper.

The traditional analysis sets the free energies of certain important species, such as O_2 and H_2, to zero, thus convolving their bond energies into the free energies of formation of all their products. For instance, $\Delta_f G^o_{\mathrm{H_2O}} = G^o_{\mathrm{H_2O}} - G^o_{\mathrm{H_2}} - 1/2G^o_{\mathrm{O_2}}$, where the energy of O_2 relative to the free atoms, $G^o_{\mathrm{O_2}} = -464$ kJ/mol, is dominated by the bond formation enthalpy of −498 kJ/mol of molecular oxygen. Therefore, while $G^o_{\mathrm{H_2O}}$ reflects only the enthalpy and entropy of H_2O itself, $\Delta_f G^o_{\mathrm{H_2O}}$ depends also on the bond strengths of H_2 and O_2 and is thus not suitable for explaining which molecule stores chemical energy. While both approaches produce correct numerical results, the traditional approach has no explanatory power.

Half-Reaction Analysis. A redox reaction can be viewed as the sum of two suitably balanced half-reactions. For the sample reaction in eq 14, they are

$$\mathrm{O_2(g) + 4H^+(aq) + 4e^- \rightarrow 2H_2O(}l) \tag{16a}$$

$$\Delta_{\text{red}} G^{o\prime}_1 = 2G^o_{\mathrm{H_2O}} - (G^o_{\mathrm{O_2}} + 4G^{o\prime}_{\mathrm{H^+}}) = -1968\ \mathrm{kJ/mol} \tag{16b}$$

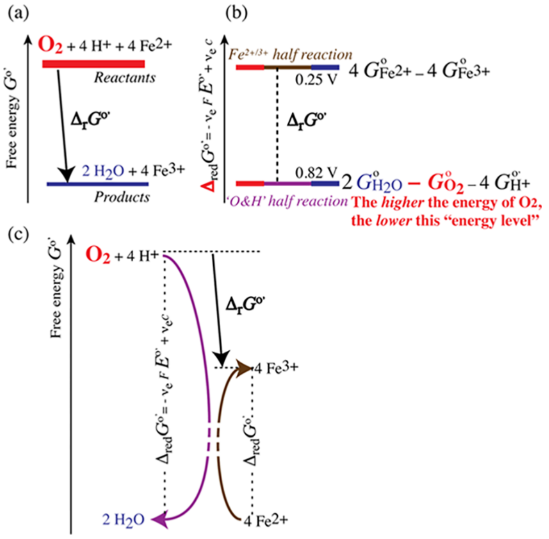
(a)
Free energy $G^{o\prime}$
O_2 + 4 H+ + 4 Fe2+
Reactants
$\Delta_r G^{o\prime}$
2 H_2O + 4 Fe3+
Products
(b)
$\Delta_{\text{red}}G^{o\prime} = -\nu_e F E^{o\prime} + \nu_e c$
$Fe^{2+/3+}$ half reaction
0.25 V
4 G^o_{Fe2+} – 4 G^o_{Fe3+}
$\Delta_r G^{o\prime}$
0.82 V
2 G^o_{H2O} – G^o_{O2} – 4 $G^{o\prime}_{H+}$
'O&H' half reaction
The *higher* the energy of O2, the *lower* this "energy level"
(c)
Free energy $G^{o\prime}$
O_2 + 4 H+
$\Delta_r G^{o\prime}$
4 Fe3+
$\Delta_{\text{red}}G^{o\prime} = -\nu_e F E^{o\prime} + \nu_e c$
$\Delta_{\text{red}}G^{o\prime}$
2 H_2O
4 Fe2+

Figure 2. Three different representations of the free energy $\Delta_r G^{o\prime}$ released in a redox reaction. The chosen example with $\Delta_r G^{o\prime} = -218$ kJ/mol is the initial reaction in eq 14 in the energy transfer chain of aerobic respiration. Here, Fe^{2+} and Fe^{3+} are meant to be incorporated into cytochrome *c*, and H^+ is in aqueous solution. (a) Reactant and product energy levels. The label "$O_2 + 4H^+ + 4Fe^{2+}$" means $G^o_{\mathrm{O_2}} + 4G^{o\prime}_{\mathrm{H^+(aq)}} + 4G^o_{\mathrm{Fe^{2+}(cyt}c)}$. (b) Half-reaction "energy levels" ("standard reduction potentials") as widely used in biochemistry. The numerical values of the two levels on the absolute scale (G^o relative to the free atoms) are −1748 and −1968 kJ/mol at pH 7 (see also Figure S1). The figure highlights that the higher the energy $G^o_{\mathrm{O_2}}$ of O_2, the lower the associated half-reaction energy level. Note that the vertical axis shows an energy difference rather than absolute free energy and that one cannot draw an arrow pointing from reactants to products. (c) Redox energy transfer and release diagram showing the free energies of reactants and products in the half-reactions, with the low-energy species in the half-reactions aligned. The curved arrows point from specific reactants to products. Dashed lines indicate that the vertical range of the curved arrows (−1968 and +1749 kJ/mol) has been shortened for easier graphing.

$$\mathrm{4Fe^{2+}(cyt}c) \rightarrow \mathrm{4Fe^{3+}(cyt}c) + 4e^- \tag{16c}$$

$$\begin{aligned}\Delta_{\text{ox}} G^o_2 &= 4G^o_{\mathrm{Fe^{3+}(cyt}c)} - 4G^o_{\mathrm{Fe^{2+}(cyt}c)} = -\Delta_{\text{red}} G^o_2 \\ &= 1749\ \mathrm{kJ/mol}\end{aligned} \tag{16d}$$

The chemical species on the left-hand side of eqs 16a and 16c can be viewed as electron acceptors and those on the right as electron donors. The free energy $\Delta_{\text{hr}} G^{o\prime}_i$ of half-reaction *i* can be obtained in the usual way (i.e., as $\Sigma_j \nu_j G^{o\prime}_{\text{prod},j} - \Sigma_j \nu_j G^{o\prime}_{\text{reactants},j}$), except that the electrons are ignored (which is without consequence since they are only intermediates in the overall reaction). The relation to the standard reduction potential $E^{o\prime}_i$ of the half-reaction is

$$\Delta_{\text{red}} G^{o\prime}_i = -\nu_e F E^{o\prime}_i + \nu_e c \tag{17}$$

where ν_e is the stoichiometric coefficient of the electrons ($\nu_e = 4$ in our example). The constant in the last term is $c = -413$ kJ/mol for conventional $E^{o\prime}_i$ values and $c = 0$ for "absolute reduction potentials on the vacuum scale".[29] Based on eq 17, it is common in biochemistry to refer to $E^{o\prime}_i$ (in volts) instead of $\Delta_{\text{red}} G^{o\prime}_i$ (in kJ/mol) (see also the Supporting Information).

Since the half-reactions add to give the overall reaction, their free energies add to give the overall free energy of reaction

$$\Delta_r G^{o'} = \Delta_{\text{red}} G_1^{o'} + \Delta_{\text{ox}} G_2^{o'} = \Delta\Delta_{\text{red}} G^{o'} \tag{18a}$$

$$\Delta_r G^{o'} = \Delta_{\text{red}} G_1^{o'} - \Delta_{\text{red}} G_2^{o'} = -\nu_e F(E_1^{o'} - E_2^{o'}) \tag{18b}$$

$$\begin{aligned}\Delta_r G^{o'} &= 2G^{o}{}_{\text{H}_2\text{O}} + 4G^{o}{}_{\text{Fe}^{3+}(\text{cyt}c)} \\ &-(G^{o}{}_{\text{O}_2} + 4G^{o'}{}_{\text{H}^+} + 4G^{o}{}_{\text{Fe}^{2+}(\text{cyt}c)})\end{aligned} \tag{18c}$$

where eq 18c reproduces eq 15. According to eq 18b, the free energy of reaction can be obtained as the difference in the energies of the half-reactions (when both are written as reductions). In a graph of half-reaction free energies $\Delta_{\text{red}}G^{o\prime}{}_i = -\nu_e FE^{o}{}_i + \nu_e c$, the "gap" between the "reduction potential free energy levels" is $\Delta_r G^{o\prime}$ (see Figure 2b and Figure S2).[6]

Note that Figure 2b is fundamentally different from Figure 2a: the upper level in Figure 2b is not the total reactant free energy but a difference between reactant and product free energies, and the lower level is not the total product energy but a difference between product and reactant energies. Consequently, an arrow from the higher to the lower level would not point from reactants to products.

A Superior Representation of Half-Reaction Energetics: Redox Energy Transfer and Release Diagrams. The two types of "energy-level" diagrams described here and exemplified in Figure 2a,b can be useful for some purposes, and indeed, one finds various examples of half-reaction energy representations (ii) in biochemistry textbooks.[1,6] However, both presentations have significant limitations. In view (i), the energetics of different reactants (O_2, $4H^+$(aq), and $4Fe^{2+}$(cyt*c*) in our example) are lumped together, and therefore, stored chemical energy is not easily attributed. In addition, half-reactions are not distinguished, even though they are often spatially separated from each other. Furthermore, for a series of reactions, it is very cumbersome that the "by-stander" reactants of future reactions or products of previous reactions need to be included (see Figure S1). The half-reaction view (ii) needs to be used with great caution; it does not show clearly where chemical energy is stored since each energy level is a difference between reactant and product energies.

To overcome these shortcomings, we propose a presentation that shows not only separate half-reactions but also the energetics of different reactants (relative to their corresponding products), giving a better indication of where chemical energy is stored. It is demonstrated in Figure 2c for our sample reaction in eq 14. The tips of the curved arrows indicate the spontaneous directions of the coupled half-reactions. The vertical reach of each curved arrow is the free energy difference $\Delta_{\text{red}}G_i^{o\prime}$ of the half-reaction. According to eq 18b, the difference of these differences is $\Delta_r G^{o}$, as shown in the figure. The diagram shows "redox energy transfer (from O_2 + $4H^+$ to Fe^{2+}) and release ($\Delta_r G^{o\prime}$)" (RETAR).

The only adjustable parameter in the RETAR diagram is the relative vertical shift of the left half-reaction versus the right half-reaction. This is discussed in more detail in the Supporting Information and Figure S3. To make it possible to read off $\Delta_r G^{o}$ directly, aligning the lowest-free energy species, as shown in Figure 2c, or the highest, as shown in Figure S3b, is advisable. Electron transfer can also be shown explicitly in the diagram (see Figure S3b).

Unlike the two energy level diagrams in Figure 2a,b, the redox energy transfer and release diagram of Figure 2c shows very clearly that the high-energy reactants storing chemical energy are $O_2 + 4H^+$. Since Fe^{2+}(cyt*c*) is lower in energy than Fe^{3+}(cyt*c*) (ripping an electron off a cation is always energetically uphill), Fe^{2+}(cyt*c*) cannot be regarded as a high-energy species. Also, the diagram makes clear that some energy is transferred from O_2 + $4H^+$ to be stored in Fe^{3+}(cyt*c*). This energy is released in the next step in the energy transfer chain as Fe^{3+}(cyt*c*) is reduced back to Fe^{2+}(cyt*c*) (see Figure 3).

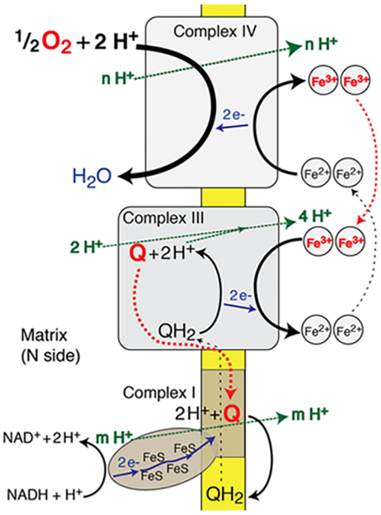

Figure 3. Schematic redox energy transfer and release diagram of aerobic respiration (equivalent to the traditional electron transfer chain, with the reactions in eqs 14, 25, and 26). The inner mitochondrial membrane (in yellow) and three protein complexes (in gray and brown) are shown schematically. Complex II, with an alternative reaction of coenzyme Q, has been omitted for clarity. The solid curved black arrows show energy transfer and release in pairs of redox half-reactions; here, a vertical free energy axis is implicit. The number of protons pumped (slanted dashed green arrows) should be proportional to the energy released (energy difference between the left and right solid curved arrows). Small blue arrows indicate electrons transferred. Energy-carrying oxidized species are highlighted in red and boldface. Motions of energy-carrying species are indicated by bold red dashed curved lines with arrowheads, while motions of their electron-carrying reduced counterparts are shown by thin dashed arrows.

Correcting Common Electron Transfer Misconceptions. In the following, we discuss common misconceptions about electron transfer. Note that the section headlines are true statements rather than the corresponding misconceptions.

The Higher the Energy of O_2, the Lower Its Half-Reaction Free Energy Level. The half-reaction energy level of the half-reaction with O_2 is low (see Figure 2b); it is in fact the lowest in the entire electron transfer chain of respiration (Figure S2a).[6] This can easily lead to the misconception that O_2 is a low-energy species.[1,3,6]

To avoid this mistake, one needs to realize that half-reaction levels are not actual energy levels of chemical species. Unlike the free energy levels in Figure 2a, half-reaction levels do not reflect a free energy or sum of free energies but rather a difference in free energies. Specifically, the energy level for the half-reaction in eq 16a/eq 16b with O_2 is

$$\Delta_{\text{red}} G_1^{o'} = 2G^{o}{}_{\text{H}_2\text{O}} - \mathbf{G^{o}{}_{O_2}} - 4G^{o'}{}_{\text{H}^+} \tag{19}$$

Due to the minus sign in front of $G^{o}{}_{O_2}$, the higher the free energy of O_2 (weaker bonding), the lower its half-reaction energy level.

For the same reason, the energy level $\Delta_{\text{red}}G^{o}{}_{F_2/2F^-} = 2G^{o}{}_{F^-} - G^{o}{}_{F_2}$ of the half-reaction $F_2 + 2e^- \rightarrow 2F^-$ is extremely low (see

Figure S2b). F_2 is undoubtedly a high-energy molecule with a weak bond (the bond formation enthalpy is only −155 kJ/mol), which results in a bond free energy of −248 kJ for 2 mol of F−F bonds, higher (less negative) than for any of the molecules in Figure 1. This simple example confirms convincingly that weaker bonding, or higher energy, of the electron acceptor lowers the half-reaction energy level.

It is true that an oxidation half-reaction is less unfavorable energetically the lower the energy of the reduced reactant's own conjugate electron acceptor (Fe^{3+}(cyt*c*) for the reactant Fe^{2+}(cyt*c*) in the half-reaction in eq 16c and overall reaction in eq 14). However, that acceptor is not initially present; it is only generated by the reaction, and electrons are not transferred to this acceptor. The actual acceptor among the reactants is always the acceptor of the other half-reaction, which is $O_2 + 4H^+$ in our example (eq 14). Since that acceptor is a reactant, the higher its energy, the more energy is released by the overall reaction.

Hydrogen, Rather than Oxygen, Is the Main Terminal Electron Acceptor. It is commonly claimed that $O_2(g) + 4H^+(aq) + 4e^- \rightarrow 2H_2O(l)$ is driven by "electron transfer to oxygen".[1,3,6] This description is not tenable. Rather, this half-reaction mostly involves electron transfer to hydrogen, similarly as in the related half-reaction

$$4H^+(aq) + 4e^- \rightarrow 2H_2(g)$$
$$\Delta_{red}G^o = -1652\ \mathrm{kJ/mol},\ \Delta_{red}G^{o'} = -1492\ \mathrm{kJ/mol} \tag{20}$$

where, undoubtedly, the electrons are transferred to hydrogen. Each H in H_2O has an effective atomic charge of +0.33e,[30] which means that 67% of an electron charge compensates the proton charge (+1e − 0.67e = +0.33e) and is thus associated with hydrogen. Only 33% of the two electrons is transferred to oxygen. The charge distribution is shown schematically in Figure S4. In other words, significantly more of the electrons are transferred to hydrogen than to oxygen.

The same is true for other molecules considered as electron acceptors. In the reaction of coenzyme Q

$$Q + 2H^+(aq) + 2e^- \rightarrow QH_2 \tag{21}$$

the electrons are primarily accepted not by Q but by H^+ since, for each of the two O−H bonds formed, the majority of one electron in the covalent bond will become associated with H. While the electrons are not primarily transferred to Q, it would be correct to say that the electrons are (completely) transferred into QH_2.

The notion of "electron transfer to oxygen" is a misguided attempt based on a mistaken 1920 postulate of "liberation of energy in the interdisplacement ... of electrons between atoms or molecules"[31,32] to explain why the half-reaction in eq 16a with O_2 ($\Delta_{red}G^o = -2128$ kJ/mol, $\Delta_{red}G^{o\prime} = -1968$ kJ/mol) is more energetically favorable than the reaction in eq 20 without O_2. In reality, the difference of −476 kJ/mol between the two half-reactions is mostly due to the high energy of the weak double bond in O_2[12,20] relative to two bonds in H_2O (by −429 kJ/mol).

An Electron Waterfall? A common representation of the electron transport chain in aerobic respiration is as an "electron waterfall".[6,24] Here, electrons are thought to move from a high-energy donor through other species (acceptors? donors?) to a low-energy terminal acceptor, O_2. This picture, a flawed interpretation of Figure 2b or Figure S2a, is wrong in many respects. To see how, we consider again the example of $O_2 + 4H^+(aq) + 4Fe^{2+}(\text{cyt}c) \rightarrow 2H_2O + 4Fe^{3+}(\text{cyt}c)$. Since some of the errors have already been discussed, the analysis can be brief.

First, the electrons are not transferred to O_2 but are mostly accepted by $4H^+$ and end up in $2H_2O$. Second, the simplistic electron energy "waterfall" picture[6,24] incorrectly proposes that the electron donor Fe^{2+}(cyt*c*) is a high-energy species responsible for the electron flow. However, donation of electrons by Fe^{2+} is actually energetically uphill since it requires ionization; this is correctly shown in the RETAR diagram (Figure 2c). Third, it is an indisputable fact (eq 19) that the "drop in the waterfall", $\Delta_rG^{o\prime}$, is greater in magnitude if the electron acceptors $O_2 + 4H^+$ are not of low but of high energy (weak bonding).

Even if the incorrect notion about electron transfer to a low-energy acceptor is given up, the electron waterfall picture is still easily misinterpreted. It is enticing to view it as a diagram of the free energy per electron, that is, the electron electrochemical potential, in half-cells associated with the standard reduction potentials. However, this is highly problematic, being equivalent to the difficult problem of determining the real (not "vacuum") half-cell or single-electrode potential.[29] The electrochemical potential of an electron generated in a half-reaction depends on the "medium" (metal electrode or intermediate acceptor molecule) in which the electron is deposited; for a metal electrode, the relevant energy would be the work function or the true Fermi energy.[29] A simple example where electrons in electrodes with different standard reduction potentials have the same free energy is presented in Figure S5.

In the end, the use of standard reduction potentials is just a device to summarize and visualize the crucial free energy change Δ_rG^o in redox reactions as a simple difference according to eq 18b. When fundamental insight is to be conferred, a standard reduction potential diagram as in Figure 2b or Figure S2 is inferior to the RETAR diagram introduced in Figure 2c, which provides much more information about the energetics of the species involved in the half-reactions.

The Reduced "Electron-Carrying" Species Do Not Carry the Energy. In the standard description of biochemical energetics, electron flow is implicitly presented as a stand-in for energy flow.[1−3,6] Here, we show that the electron-carrying species in the mitochondrial electron transfer chain do not carry the energy; rather, their oxidized counterparts do.

Fe^{2+} in cytochrome *c* carries an electron from complex III in the inner mitochondrial membrane to complex IV, where it gives off the electron (into H_2O) forming Fe^{3+}(cyt*c*). Fe^{2+} is lower in energy than the more ionized Fe^{3+}, so it is Fe^{3+}(cyt*c*) that carries energy from complex IV to complex III, where Fe^{3+}(cyt*c*) is the high-energy species in the next redox reaction (see Figure 3). In the Supporting Information, the corresponding analysis is presented for ubiquinone (coenzyme Q) carrying energy and its fully reduced counterpart, QH_2, carrying electrons between complex III and complexes I or II in the mitochondrial membrane.

Electrons transfer energy only if they move relative to the associated positive ions. So, while electrons moving through a wire produce an electric current carrying energy, electrons moving with a wire through a field-free space do not (their kinetic energy is negligible due to their small mass compared to the nuclei). Similarly, the "extra" electrons in diffusing neutral QH_2 do not give rise to an electric current and do not carry energy; due to its stabilization by an additional bond, QH_2 can actually be considered as being lower in energy than Q.

Electron Transfer and Energy Release. *Electron Transfer and Bond Energies.* Electron transfer, usually associated with standard reduction potentials $E^{o\prime}$, has been greatly emphasized in the traditional description of the "terminal" processes of respiration and of the primary light reaction of photosynthesis. However, closer inspection shows that electron transfer is a description, not an explanation, since its "driving force" is just empirical $E^{o\prime}$ values or nebulous "affinities for electrons"[1] that are not quantified in terms of atomic or molecular properties. The reduction potentials are simply taken as given properties of molecules or ions, while they, in fact, derive from bond, ionization, and hydration energies of conjugate oxidized and reduced species, as we have shown for galvanic cells and batteries.[33]

The electron transfer picture may be appropriate for a few simple half-reactions involving only a change in the charge of a hydrated ion (e.g., from Fe^{3+} to Fe^{2+}). However, if the chemical bonding changes, for example, in $O_2(g) + 4H^+(aq) + 4e^- \rightarrow 2H_2O(l)$, the difference in bond energies of reactants and products often makes the dominant contribution to the energetics and drives the electron flow.

The easily misunderstood phrase that "electron transfer drives"[16] redox processes should be avoided. The electrons do not drive the underlying exergonic redox reaction but, on the contrary, are driven by the free energy difference between reactants and products. As an example, consider a fuel cell, where the electron flow is a secondary effect of a redox reaction, not its driving force. Nevertheless, just like a running fuel cell that can charge a battery, the electron flow in respiration can drive other endergonic redox processes, which eventually leads to ATP production.

Local but Not Global Electron Flow. The purpose of electron transfer at a membrane is to control reactions with very negative $\Delta_r G$, minimizing release of the energy as heat. This is achieved by reactants remaining separated by the membrane, which is analogous to the separation of half-cells in fuel cells or many galvanic cells. For instance, if the $Cu^{2+}(aq)$ ions in a Daniell cell were allowed to react directly with the Zn(s) electrode, then no electrical work but just heat would be produced as $Zn^{2+}(aq)$ and Cu(s) are formed.

In biochemistry textbooks, electron transfer is often represented in cartoons suggesting long-range electron flow, for instance, to NADH in glycolysis and the citric acid cycle.[1,3,6] In reality, electrons flow only over a few nanometers in most biochemical processes; the diffusion of neutral reduced molecules does not produce an energetically relevant electron flow, as pointed out above. Instead, chemical energy is transported in weak bonds, in ions, or in potential bonds yet to be formed and is often higher in molecules or ions with fewer electrons. The purpose of what has been called "electron transfer" is often arguably hydrogen transfer, for example, from glucose to NADH in glycolysis or from NADPH to CO_2 in photosynthesis of biomolecules.

The Electron Transfer Chain in Aerobic Respiration. In the following, we analyze the well-studied and relatively simple terminal steps of aerobic respiration, which for historical reasons is referred to as "oxidative phosphorylation".[1−3,6] Figure 3 shows a schematic of the electron transfer processes at the inner mitochondrial membrane, with a level of detail similar as in introductory biochemistry textbooks.[1−3,6] The electron transfer can be summarized as

$$\text{NADH} \rightarrow \text{QH}_2 \rightarrow 2\,\text{Fe}^{2+}(\text{cyt}c) \rightarrow \text{H}_2\text{O} \tag{22}$$

This simple version of the chain includes only species that carry the electrons; why O_2 does not show up is discussed in the Supporting Information. (In addition, it has already been pointed out above that electrons are transferred into H_2O but not primarily to oxygen.) The corresponding energy transfer chain listing the high-energy oxidized species is

$$\begin{aligned} &\text{O}_2 + 4\text{H}^+(\text{aq}) \rightarrow 4\text{Fe}^{3+}(\text{cyt}c) \rightarrow 2\text{Q} + 4\text{H}^+(\text{aq}) \\ &\quad \rightarrow 2\text{NAD}^+ + 2\text{H}^+(\text{aq}) \end{aligned} \tag{23}$$

(with twice larger stoichiometric coefficients than in eq 22). Note that the main purpose of the chain is not so much transfer of electrons or energy to the next species in the chain but rather controlled release of energy in the process ("during the arrows").

Energetics of the Aerobic Respiration Chain. The natural starting point for analyzing the flow of chemical energy in aerobic respiration (see Figure 3) is the reaction involving O_2, eq 14, which releases $\Delta_r G^{o\prime} = -218$ kJ/mol at pH 7 for $\nu_{O_2} = 1$, converting $4Fe^{2+}(cytc)$ to $4Fe^{3+}(cytc)$. The released chemical energy can be attributed mostly to molecular O_2, specifically its relatively weak double bond. This attribution is confirmed when we compare the reaction in eq 14 with the (hypothetical) analogous reaction without oxygen

$$\begin{aligned} &4\text{Fe}^{2+}(\text{cyt}c) + 4\text{H}^+(\text{aq}) \rightarrow 4\text{Fe}^{3+}(\text{cyt}c) + 2\text{H}_2 \\ &\quad \Delta_r G^{o\prime} = +256\ \text{kJ/mol} \end{aligned} \tag{24}$$

In this case, the reaction is not even exergonic and will not occur spontaneously. The difference in the free energies of the two reactions is close to the −418 kJ for 1 mol of O_2 in combustion.[12]

While in the reaction in eq 14 about half of the energy of O_2 is given off to eventually generate ATP, the other half is used to generate $Fe^{3+}(cytc)$, a high-energy electron acceptor that drives the energetics of the next reaction in the chain, in complex III

$$\begin{aligned} &\text{QH}_2 + 2\text{Fe}^{3+}(\text{cyt}c) \rightarrow 2\text{Fe}^{2+}(\text{cyt}c) + \text{Q} + 2\text{H}^+(\text{aq}) \\ &\quad \Delta_r G^{o\prime} = -30\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = -33\ \text{kJ/mol} \end{aligned} \tag{25}$$

(for $\Delta_r G^{o\prime}$ in eq 25, see ref 16).

This generates the moderately-high-energy coenzyme Q, which, in complex I, drives

$$\begin{aligned} &\text{Q} + \text{H}^+(\text{aq}) + \text{NADH} \rightarrow \text{QH}_2 + \text{NAD}^+(\text{aq}) \\ &\quad \Delta_r G^{o\prime} = -81\ \text{kJ/mol},\ \Delta_r G^{o\prime\prime} = -69\ \text{kJ/mol} \end{aligned} \tag{26}$$

due to formation of two fairly strong O−H bonds and aromatic stabilization in QH_2. (If the $4Fe^{3+}(cytc)$ in the earlier reactions in eqs 14 and 24 are to be matched, all stoichiometric coefficients and $\Delta_r G^{o\prime}$ values should be doubled in eqs 25 and 26.) The redox energy transfer and release diagram in Figure 3 with NADH near the bottom confirms our earlier conclusion that calling NADH a high-energy molecule[27,28] is not warranted. The energy released in the chain of reactions in eqs 14, 25, and 26, equivalent to the reaction in eq 6, to eventually produce ATP can mostly be traced back to O_2.

Reconsidering the Number of Protons Pumped by the Energy of O_2. According to the widely accepted chemiosmotic theory,[1−3,6] the free energy released in the reactions of aerobic respiration is used to pump protons across the inner mitochondrial membrane. Textbooks claim that dioxygen in the reaction in eq 14, balanced with $\nu_{O_2} = 1$ and $\Delta_r G^{o\prime} = -218$ kJ/mol, pumps only 4 + 4/2 = 6 protons (ref 1, p 742) while 2Q + $4H^+(aq)$ + 2NADH (eq 26) with $\Delta_r G^{o\prime} = -162$ kJ/mol pumps as many as 8 + 2/2 = 9 protons. (Here, we count protons as half if they are removed on one side of the membrane but not transported across.)

Since the reaction of O_2 releases 218/162 = 1.3 times more energy than that of 2Q, it should pump 12 rather than 6 protons if the reaction of 2Q pumps $9H^+$. The energy per mole of protons pumped is ~20 kJ/mol,[16] so more than 6 protons should be pumped by the free energy change of −218 kJ/mol. It is unlikely that the magnitude of 218 kJ/mol is an overestimate, given that eq 14 has a major contribution from the large bond energy change from O_2 to H_2O, which is not subject to variation due to biochemical conditions. In fact, Alberty's data[14] give an even larger magnitude of $\Delta_r G^{o\prime\prime} = -229$ kJ/mol for the reaction in eq 14.

If only 6 protons were pumped by the O_2 reaction, then the corresponding free energy absorption would be less than 140 kJ/mol, and the rest of the released 229 kJ/mol, >90 kJ/mol, would have to be wasted as heat or taken up by another endergonic process. While this discrepancy exists simply based on the $\Delta_r G^{o\prime}$ values, independently of our assignment of the energy to specific molecules, recognizing O_2 as the main carrier of chemical energy makes it clear that the large amount of energy released by the reaction of O_2 is to be expected and that the endergonic processes that it drives, proton pumping or otherwise, have not been fully identified.

Glycolysis and Citric Acid (Krebs) Cycle. The discussion so far has focused on energy release from O_2 in a chain of reactions in eqs 14, 25, and 26 with NADH as the terminal reactant, producing NAD^+. NADH is regenerated by the reaction of NAD^+ with the hydrogen atoms released from glucose as it gets converted to CO_2 in the course of glycolysis, decarboxylation of pyruvate to acetyl plus CO_2 and NADH, and the citric acid (or Krebs) cycle.[1−3,6] The net reaction (without ADP) is shown in eq 10g, with $\Delta_r G^{o\prime} = -341$ kJ/mol and $\Delta_r G^{o\prime\prime} = -307$ kJ/mol, which results in phosphorylation of ~4 ADP molecules to ATP.[1] For reference, respiration of the same amount of glucose with oxygen releases nearly 9 times more energy (see eq 1) and can generate correspondingly more ATP. The net reaction in glycolysis (without ~2 ADP molecules) from glucose to pyruvate and NADH is given by eq 3a, with modest $\Delta_r G^{o\prime} = -147$ kJ/mol[16] and $\Delta_r G^{o\prime\prime} = -153$ kJ/mol. Then, by difference, the citric acid cycle (plus pyruvate decarboxylation) accounts for meager $\Delta_r G^{o\prime} = -194$ kJ/mol and $\Delta_r G^{o\prime\prime} = -154$ kJ/mol. This disproves a textbook claim (ref 10, Table 18-1) that the citric acid cycle produces a large number of ATP molecules.

The Calvin Cycle. The Calvin cycle in the dark reaction of photosynthesis converts three molecules of CO_2 to glyceraldehyde 3-phosphate, with hydrogen provided by NADPH and phosphate by ATP.[1−3,6] As shown in the Supporting Information, the net reaction (leaving out $8ATP + 8H_2O$) can be written as

$$3CO_2 + 6\text{“}H_2\text{”}_{NADPH} + ATP \rightarrow C_3H_5O_3PO_3H + 3H_2O + ADP \quad \Delta_r G^{o\prime\prime} = +139\ \text{kJ/mol} \tag{27}$$

If organic molecules contained a lot of chemical energy, their production from CO_2 should require much energy input, comparable to the 2875 kJ/mol of eq 1 run in reverse. In reality, as eq 27 shows, only ~280 kJ/mol is required (on a six-carbon basis). The bond energies in Figure 1b visualize this small energy change. When the $8ATP + 8H_2O$ is included in the overall Calvin cycle reaction, the cycle is exergonic ($\Delta_r G^{o\prime\prime} = -157$ kJ/mol), as required.

Synopsis. *The Traditional Description of Bioenergetics Is Incorrect.* Some readers who have taught the traditional version of bioenergetics for many years may be inclined to declare that it is equivalent to the new view introduced here. However, this is not consistent with the evidence. The traditional explanation assuming high-energy fuels and electron transfer to oxygen fails to provide a comprehensive, consistent explanation of the experimental observations and therefore needs to be abandoned. These shortcomings can be highlighted by analyzing, as before, the "terminal electron transfer reaction" (eq 14), $O_2 + 4H^+(aq) + 4Fe^{2+}(\text{cyt}c) \rightarrow 2H_2O + 4Fe^{3+}(\text{cyt}c)$:

(i) The traditional explanation in terms of electron transfer to oxygen is mechanistically incorrect: the electrons are mostly transferred to hydrogen, not oxygen (see above).

(ii) The electron transfer or fuel energy description cannot explain why this reaction is highly exergonic and exothermic: no organic fuel is involved in this reaction, yet a lot of energy is produced (approximately −1350 kJ/mol per $6O_2$). Since $Fe^{2+}(\text{cyt}c)$ is lower in energy than $Fe^{3+}(\text{cyt}c)$, organisms are certainly not fueled by $Fe^{2+}(\text{cyt}c)$ and, thus, the energy released by the reaction in eq 14 unquestionably comes from O_2, a proven high-energy molecule.

(iii) Any explanation connecting electron transfer between molecules with the energy released must include bond energies. These are quantities with energy units and real explanatory power unlike standard reduction potentials in volts. The energy stored in O_2 can be attributed quantitatively to its relatively weak double bond,[12] which is less stable (higher in energy) by 250−410 kJ/mol relative to a double bond in CO_2 or a pair of single bonds in organic molecules or in H_2O (see Figure 1).

(iv) The standard reduction potential levels in the electron waterfall picture[6,24] are not energies of chemical species but differences between such energies. Therefore, they do not reveal where chemical energy is stored. Specifically, the level of the terminal half-reaction is lowered as the energy of the electron acceptors, $O_2 + 4H^+$, is increased. Both changes would increase the energy released with the electron: the acceptors are reactants, and higher reactant energy increases the energy released by a reaction.

(v) The traditional electron transfer analysis has ignored the (model-independent) large negative $\Delta_r G^{o\prime}$ value of this reaction in eq 14, which was just another meaningless empirical value, and therefore overlooked that it is incompatible with the small number of protons supposedly pumped by complex IV. Recognizing O_2 as a high-energy molecule, our analysis correctly predicts the large negative $\Delta_r G^{o\prime}$ value and highlights the factor-of-two discrepancy in the number of H^+ pumped.

The basic traditional assumption that fuel molecules contain the energy released in respiration is not supported by the facts. We have highlighted that all reactions of organic biomolecules with O_2 are highly exothermic and exergonic, even if no CO_2 or H_2O is generated, confirming that O_2 is properly considered a high-energy molecule. The high-energy fuel molecule assumption fails to explain why the supposedly high-energy fuel molecules do not show their energy in reactions without oxygen, such as fermentation. Whether glucose fragments into ethanol and CO_2 or completely rearranges its bonding when forming CO_2 and CH_4, according to eqs 2c and 2f, only −225 to −425 kJ/mol are released (of which approximately −200 kJ/mol is from entropy, not bond energies).

Ignoring these new insights and continuing to promulgate the incorrect traditional description of respiration would be akin to explaining summer as arising from Earth being closer to the sun than in winter, or day and night from the sun revolving around the Earth: the description "makes intuitive sense" and would be a good explanation if it was true, but closer inspection disproves it and a different explanation quantitatively accounting for all observations has to be accepted and taught.

Limitations of the Electron Transfer Paradigm. Electron transfer has been presented as a stand-in for energy flow in biochemical processes.[1−3,6] This needs to be reassessed. Electron flow in and of itself is of limited importance in cells compared to energy flow and atom transfer. True electron transfer involving electrons separated from atoms does not occur outside of specialized filaments over distances of more than a few nanometers; its purpose is controlled energy transfer minimizing the release of heat. Diffusion of electrons bound in reduced molecules is not diffusion of energy since the oxidized counterparts of these carriers are of higher energy. Transfer of hydrogen atoms appears to be the main benefit of many so-called electron transfer processes. Electron transfer has no true explanatory power for energy flow since it does not reveal which weak bonds initially stored the energy.

In standard biochemical pathways, there is no relevant analogy to electron flow in a metal wire producing a force on the wire in a magnetic field and, consequently, work in a motor. The only applicable macroscopic analogy may be electrons transferring chemical energy from one charging battery or fuel cell to another being charged. Proton transfer, being the driving force of ATP synthesis, seems equally relevant as electron transfer for energy transduction since energy can be stored more easily in proton concentration gradients than in distinct transferred electrons. As an example of how existing textbook presentations can be reconciled with these considerations, a revision of an introductory section on energy and electron transfer from Lehninger's biochemistry text[1] is proposed at the end of the Supporting Information.

Bond Energies as the Crucial Unifying Concept of Bioenergetics. Bioenergetics should be built on the quantifiable[12,33] principle that chemical energy is stored in relatively weak bonds and intermolecular interactions or in a high degree of ionization. The energy is released when stronger bonds form or ions are reduced. The main effect, energy stored in relatively weak bonds, can unify the description of bioenergetics from O_2 through glucose to ATP. The energy previously encrypted in standard reduction potentials given in volts can now be visualized through redox energy transfer and release diagrams. The resulting schematics can look like "typical biochemistry" (see Figure 3). Local electron transfer is only needed to control the transfer of chemical energy and avoid the release of heat. The general notion of long-range electron flow should be replaced with the concept of transfer of energy in relatively weak bonds of molecules and its release when more stable products form, which also applies to ATP and is therefore a valid overarching principle of bioenergetics from glucose and O_2 all the way to CO_2 and H_2O and back.

O_2 as the Major High-Energy Molecule in the Biosphere. We have highlighted here that molecular oxygen is the key high-energy molecule driving the energetics of aerobic organisms; in hindsight, it may be surprising that this had not been obvious. The relatively weak double bond of O_2[12,22] (for details on the bonding, see the Supporting Information) provides a simple molecular explanation for the fact that all reactions of organic molecules with O_2 release a lot of energy (250−480 kJ per mole of O_2), which is four to eight times more on a per-mass basis than most other reactions, including those involving ATP. This explains the drastic difference between aerobic and anaerobic respiration energetics, which had remained without explanation in the traditional presentation. It makes it obvious, for instance, why, without O_2, the conversion of glucose and water to CO_2 and NADH releases only little energy. Textbooks have presented the 30 or 57 kJ/mol from ATP hydrolysis as noteworthy while giving the much larger >400 kJ released per mole of O_2 only a passing reference. This is no longer tenable. O_2 plays a simple central role in bioenergetics. It also appears in other cases where organisms require a lot of energy, for instance, in the generation of cold light.[1]

O_2 as the crucial high-energy molecule explains the well-known fact that large complex multicellular life became possible only with a sufficiently high concentration of O_2 in the atmosphere. Accordingly, humans can survive for a few weeks without food and a few days without water but only a few minutes without dioxygen. Not only most animals but also plants during the night and at their roots require the chemical energy of O_2 for survival. O_2 in the atmosphere can be considered as communal storage, by plants, of chemical energy derived from solar photons. The different roles of photosystems I and II in plants resulting from the high energy of O_2 will be explained in a future paper about the energetics of photosynthesis.

■ CONCLUSIONS

We have provided an intuitive and quantitative description of biochemical energetics based on chemical energy stored mostly in weak bonds, showing that O_2 with its relatively weak double bond is the molecule with the highest energy per bond in biochemistry. The analysis properly explains why O_2 makes aerobic respiration highly exergonic. This obvious fact has, quite surprisingly, not been explained in the traditional presentation of biochemical energetics, which did not recognize the high-energy role of O_2 but instead assumed that the chemical energy powering complex organisms is stored in the bonds of glucose, NAD(P)H, or reduced molecules. Then, reactions breaking down these fuel molecules should release the energy supposedly stored in their bonds, but they actually give off little energy in the absence of O_2. Accordingly, the standard picture of energy-rich fuel molecules cannot explain why fermentation or breakup of glucose into small molecules produces only little ATP. The amounts of energy released or taken up during the Krebs and Calvin cycles are similarly unremarkable. Highly reduced molecules have even less chemical energy than glucose; they only unlock the energy of more O_2 than O-containing molecules of similar mass do. Methanogens producing energy by generating methane, a highly reduced molecule, as a low-energy product directly refute the claim that reduced molecules in biological organisms have a high energy content.

The energy stored and released by O_2 has been completely obscured in the conventional description of respiration, which is given in terms of electron transfer and standard reduction potentials, which are energy differences indirectly and non-intuitively expressed in volts and with a sign switch. O_2 has been called the low-energy "terminal electron acceptor" for spent electrons. We have exposed the underlying misconception by showing that a higher free energy of the electron acceptors (O_2 +

$4H^+$) lowers the half-reaction "free energy level" associated with the standard reduction potential.

In the first step of aerobic respiration, the energy of O_2 is used partly to help generate the H^+ gradient subsequently exploited in ATP synthesis and partly to generate a high-energy intermediate, Fe^{3+} in cytochrome *c*. This can be represented convincingly in a redox energy transfer and release diagram. The analysis highlights that the free energy released by the reaction of O_2 must pump a larger number of protons than conventionally claimed or be coupled to another endergonic process. Since this reaction of O_2 does not involve an organic molecule, it confirms that organic fuel is not the origin of the energy released. The cascade of subsequent reactions further utilizes the energy originally stored in O_2 to produce ATP. It has been described as an electron transfer chain, but arguably, not the electron-carrying reduced molecules or ions but their oxidized counterparts carry the energy. Electron transfer is useful for controlled local energy release, but its relation to long-range energy transport in neutral molecules is tenuous; a bond energy analysis is more meaningful. To present bioenergetics correctly, biochemistry textbooks will need to be revised significantly.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03352.

> Analyses of combustion energetics irrespective of the bond order of O_2; a review of bonding in O_2; a table of thermodynamic energies for biochemistry; the origin of data for Table S1 and Figure 1; conventional versus meaningful individual free energies of ions; evaluating free energies of reactants and products; discussions of "weak bonds to strong bonds" and of the small entropy of combustion; reduced molecules and their reaction energetics; the analogy of NAD(P)H + H^+ and H_2; O_2 in the last or first step of respiration; more electron transfer to hydrogen than oxygen; presenting the electron transfer chain of aerobic respiration correctly, with reduced "electron-carrying" molecules not carrying the energy; "half-reaction free energy levels" and standard reduction potentials versus electron energies; energetics of the Calvin cycle; a review of biochemical energy in textbooks; and an example of correcting bioenergetics in a textbook (PDF)

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Notes

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